REMARKS

Status of the Claims

Claims 1-20 are pending in the present application. Claims 9-17 are withdrawn as directed to a non-elected invention. Claims 1 and 2 are amended to specify "90% identity" and hybridization conditions of "0.2 x SSC, 0.1% SDS, 65°C." Support for these amendments is found throughout the application as originally filed including, *e.g.*, on page 15, paragraph 1. Claim 18 is amended to clarify that the acronym FPP refers to farnesyl pyrophosphate. Support for this amendment is found, *e.g.*, on page 17, paragraph 4, in the originally filed application. Claims 19 and 20 are new. Support for new claims 19 and 20 is found, *e.g.*, in original claims 1-3, and on page 15, paragraph 1, in the originally filed application. The claims are amended without prejudice or disclaimer. No new matter is entered by way of this amendment. Reconsideration is respectfully requested.

Statement of Substance of Interview

Applicants and Applicants' representative thank the Examiner for extending the courtesy of an interview on September 1, 2009. The substance of the interview is essentially as described in the interview summary, which issued in the instant application on September 14, 2009.

Objections to the Claims

Claim 18 is objected to for specifying the acronym "FPP." Claim 18 is amended to clarify that FPP refers to farnesyl pyrophosphate. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

Rejections under 35 U.S.C. § 112, 1st paragraph

Enablement

Claims 1-8 and 18 comply with the enablement requirement

Claims 1-8 and 18 remain rejected under 35 U.S.C. § 112, 1st paragraph, as allegedly failing to comply with the enablement requirement, *see* Office Action, pages 4-15. In the

instant Action, the Examiner reiterates his reasons for rejecting the claims as set forth in the November 11, 2008, Office Action. In response to Applicants' amendment and reply of February 18, 2009, the Examiner alleges that, as interpreted, the claims are directed to random variant and mutant peptides having β-ionone ring-2-hydroxylase activity. Applicants respectfully traverse.

Although Applicants do not agree that the instant application fails to provide enablement support for the present claims, the claims are amended in an effort to expedite prosecution. In particular, amended independent claims 1 and 2 specify that the described peptides consist of an amino acid sequence having a 90% or more identity with the amino acid sequence as shown in SEQ ID NO: 4 or are encoded by a DNA that hybridizes to the full complement of SEQ ID NO:3 under stringent conditions of about 0.2X SSC, 0.1% SDS, 65°C.

Applicants submit that the present application enables the instant claims. An ordinary artisan would have recognized from the guidance in the present application that certain carotenoid-containing bacteria are likely to contain a sequence having the specified hydroxylase activity. Such a sequence could be identified by comparing the sequences from the bacteria to SEQ ID NO: 3 or SEQ ID NO: 4 as described in the instant claims. That is, the present application teaches that nucleic acid sequences, which hybridize to SEQ ID NO: 3 at 0.2 X SSC, 0.1% SDS at 65°C, or amino acid sequences, which share 90% or more identity to SEQ ID NO: 4, are likely to have β-ionone ring 2-hydroxylase activity, *see e.g.*, pages 14-15 in the present application.

Further, Applicants direct the Examiner's attention to Tao *et al.*, *Gene*, 2006, 379: 101-108, ("Tao", of record, as cited in the Office Action of November 11, 2008). Tao, which was published subsequently to the priority date of the instant invention, describes sequences having ionone ring-2-hydroxylase activity (referred to as CrtG in Tao). Tao describes an enzyme from a carotenoid-containing bacteria, *i.e.*, *Brevundimonas vesicularis*, which has ionone ring-2-hydroxylase activity, and which shares only 79% sequence identity with instant SEQ ID NO: 4. Tao further describes an enzyme from *Brevundimonas aurantiaca*, which shares 99% identity with SEQ ID NO: 4 and also has ionone ring-2-hydroxylase activity. These enzymes are able to hydroxylate the carotenoid, zeaxanthin.

A more recent report further demonstrates that a sequence sharing even less sequence identity with instant SEQ ID NO: 4 is also capable of carotenoid hydroxylation at the position 2 carbon of a β-ionone ring. Iwai *et al.*, *Plant cell. Physiol.*, 2008, 1678-1687, (abstract enclosed), describe a functional sequence from the cyanobacterium, *Thermosynechococcus elongates*, which shares only 41% identity with instant SEQ ID NO: 4. The function of the sequence was initially ascertained by a homology search with SEQ ID NO: 4 and verified using disruptant mutants, *see* Iwai, abstract.

New claims 19 and 20 also comply with the enablement requirement

Further, Applicants submit that new claims 19 and 20 are also enabled by the present application. New claims 19 and 20 specify that the described peptides are isolated from a naturally occurring bacterium and consist of an amino acid sequence having 90% or more identity with the amino acid sequence depicted in SEQ ID NO: 4, or which are encoded by a DNA that hybridizes to the full complement of SEQ ID NO: 3 under stringent conditions of about 0.2 x SSC, 0.1% SDS, 65°C, wherein the bacterium is capable of introducing a hydroxyl group at the position 2 carbon of a β-ionone ring.

In addition to the argument set forth above, Applicants submit that an ordinary artisan would have recognized that a naturally occurring sequence, obtained from a naturally occurring bacterium capable of introducing a hydroxyl group at the position 2 carbon of a β-ionone ring, which is highly homologous to the described sequences, is predictably a functional variant of SEQ ID NO: 4. Due to evolutionary constraints, a naturally occurring sequence from a bacterium demonstrating the described hydroxylating function is unlikely to be a non-functional random variant.

Based upon the foregoing, Applicants believe that the claimed genus of polypeptides and sequences encoding polypeptides is adequately described by the instant disclosure. An ordinary artisan may use SEQ ID NO: 4 or SEQ ID NO: 3 to isolate the polynucleotides and polypeptides having the specified activity from a carotenoid-containing bacteria, as described in the instant application. Withdrawal of the rejection is respectfully requested.

Written Description

Claims 1-8 and 18 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement, *see* Office Action, pages 15-22. Specifically, the Examiner states that the present application fails to specify any structure-function correlation. That is, the Examiner states that the present application fails to describe the amino acid sequences that are responsible for the catalytic domains, the binding domains, and/or the core motifs involved in the specified biological activity of the claimed enzymes, *see* Office Action, page 18.

Although Applicants do not agree that the instant claims fail to comply with the written description requirement, the claims are amended in an effort to expedite prosecution. As noted above, claims 1 and 2 are amended to specify that the described peptides have β-ionone ring-2-hydroxylase activity and consist of an amino acid sequence having 90% or more identity with the amino acid sequence as shown in SEQ ID NO: 4 or are encoded by a DNA that hybridizes to the full complement of SEQ ID NO:3 under stringent conditions of about 0.2 X SSC, 0.1% SDS, 65°C.

Applicants submit that amended claims 1 and 2 comply with the written description requirement. An ordinary artisan could have envisioned all of the sequences having 90% identity or more to SEQ ID NO:4 or which hybridize under the described stringency conditions to SEQ ID NO: 3. Further sequences that are homologous to SEQ ID NO: 4 also share β-ionone ring 2-hydroxylase activity, as evidenced by Tao and Iwai, above.

New claims 19 and 20 also describe the sequence identity and hybridization conditions specified for amended claims 1 and 2. In addition, new claims 19 and 20 ascribe the function, *i.e.*, the β-ionone ring-2-hydroxylase activity, to the bacterium, instead of the claimed sequences. Accordingly, claims 19 and 20 are drafted analogously to claim 1, Example 11A, in the written description guidelines, *see* www.uspto.gov/web/menu/written.pdf. Claim 1 of Example 11A describes sequences sharing 85% amino acid sequence identity with a sequence described in a hypothetical application. No function is ascribed to the claimed sequences. Claim 1 of Example 11A was recognized as complying with the written description

requirement. Analogously, instant claims 19 and 20 also comply with the written description requirement.

CONCLUSION

In view of the above amendment and remarks, Applicants believe that the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact L. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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2,2′-β-Hydroxylase (CrtG) is Involved in Carotenogenesis of Both Nostoxanthin and 2-Hydroxymyxol 2′-Fucoside in *Thermosynechococcus elongatus* Strain BP-1

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We identified the molecular structures, including the stereochemistry, of all carotenoids in *Thermosynechococcus elongatus* strain BP-1. The major carotenoid was β-carotene, and its hydroxyl derivatives of (3*R*)-β-cryptoxanthin, (3*R*,3′*R*)-zeaxanthin, (2*R*,3*R*,3′*R*)-caloxanthin and (2*R*,3*R*,2′*R*,3′*R*)-nostoxanthin were also identified. The myxol glycosides were identified as (3*R*,2′*S*)-myxol 2′-fucoside and (2*R*,3*R*,2′*S*)-2-hydroxymyxol 2′-fucoside. 2-Hydroxymyxol 2′-fucoside is a novel carotenoid, and similar carotenoids of 4-hydroxymyxol glycosides were previously named aphanizophyll. Ketocarotenoids, such as echinenone and 4-ketomyxol, which are unique carotenoids in cyanobacteria, were absent, and genes coding for both β-carotene ketolases, *crtO* and *crtW*, were absent in the genome. From a homology search, the Tlr1917 amino acid sequence was found to be 41% identical to 2,2′- β-hydroxylase (CrtG) from *Brevundimonas* sp. SD212, which produces nostoxanthin from zeaxanthin. In the *crtG* disruptant mutant, 2-hydroxymyxol 2′-fucoside, caloxanthin and nostoxanthin were absent, and the levels of both myxol 2′-fucoside and zeaxanthin were higher. Therefore, the gene has a CrtG function for both myxol to 2-hydroxymyxol and zeaxanthin to nostoxanthin. This is the first functional identification of CrtG in cyanobacteria. We also investigated the distribution of *crtG*-like genes, and 2-hydroxymyxol and/or nostoxanthin, in cyanobacteria. Based on the identification of the carotenoids and the completion of the entire nucleotide sequence of the genome in *T. elongatus*, we propose a biosynthetic pathway of the carotenoids and the corresponding genes and enzymes.

Keywords: Carotenogenesis — Carotenoid — *crtG* — Cyanobacterium — Nostoxanthin — *Thermosynechococcus elongatus*.

Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography.

Introduction

Thermosynechococcus elongatus strain BP-1 is a unicellular, thermophilic cyanobacterium, first isolated at the Beppu Hot Springs in Japan (Yamaoka et al. 1978). This strain is suggested to have branched at a very early stage in the evolution of cyanobacteria, based on the 16S rRNA sequence (Honda et al. 1999). It has become a new model cyanobacterium for genetic and physiological studies due to improved techniques for genetic engineering (Iwai et al. 2004, Onai et al. 2004, Iwai et al. 2007) and the completion of sequencing of its genome (Nakamura et al. 2002). The crystal structures of its PSI and PSII reaction center complexes have been successfully determined, by taking advantage of its thermostability (Jordan et al. 2001, Kamiya and Shen 2003, Ferreira et al. 2004, Loll et al. 2005). PSI and PSII perform light-induced electron transfer and water-splitting reactions, leading to the formation of molecular oxygen, NADPH and the transmembrane H^+ gradient. The components of PSI from cyanobacteria include 12 membrane-spanning subunits and 127 cofactors, containing 22 carotenoids. The structure of PSI has been determined at a 2.5 Å resolution by X-ray crystallographic analysis of PSI isolated from the thermophilic cyanobacterium, *T. elongatus* (Jordan et al. 2001). The components of PSII from cyanobacteria include 16–17 membrane-spanning subunits and >70 cofactors containing carotenoids. The structure of PSII has been determined at a 3.8–3.0 Å resolution by X-ray crystallographic analysis of PSII isolated from two

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